

Abstracts

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Station Collaborators'
Conference on
Food Flavor**

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★ 1967 ★**

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This conference on food flavors was one of a series of annual collaborators' conferences organized by the regional utilization divisions of the Agricultural Research Service, U. S. Department of Agriculture. The collaborators are staff representatives of the State Agricultural Experiment Stations in each of the four regions. To assure depth and breadth in subject matter, a single area of important research is selected for each conference.

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WELCOMING REMARKS

P. A. Wells

Eastern Utilization Research and Development Division

We are again happy to welcome you, our collaborators in the Eastern Region Experiment Stations, to this annual conference. This is something of an anniversary for us, for it was just 20 years ago, in 1947, that this fruitful series of conferences was initiated. We are proud of this unbroken succession of meetings which has provided a valuable forum for the exchange of information on a variety of scientific subjects related to agriculture.

This is the second time in this series that your Experiment Station directors and I have agreed on flavor as a conference theme. Many of you will no doubt recall the stimulating session we had here five years ago on the flavor and texture of foods.

Much has happened in this fast-moving field in the past five years, and we trust that the papers you will be hearing today and tomorrow will bring you up-to-date and give you an opportunity to discuss recent developments in flavor chemistry and evaluation with the experts who have agreed to participate in this conference.

I want to express a special appreciation to George Nutting, Byron Webb, Jack White, and Bill Sulzbacher for their work in arranging our program for this meeting.

MECHANISM OF ODOR PERCEPTION

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Olfaction can be compared by analogy to a system consisting of a gas chromatograph linked to a mass spectrometer linked to a computer. The odorous stimulus is detected, separated, identified, and analyzed. The analogy would be complete if the computer formed a feedback system with the gas chromatograph, thus controlling the amount of stimulus taken up. The brain controls the rich network of blood vessels of the nasal mucosa and can reduce or increase the flow of stimulus to the olfactory area by varying the blood supply to the mucosa.

Chemoreception involves response to stimuli through the taste buds (sense of taste) and the olfactory nerve (sense of smell). However, it has been shown that response can also occur through the common chemical sense; there are many free nerve endings in the throat and in both the olfactory and non-olfactory areas of the nose that respond to stimuli. Thus smell may be due to the combined activity of olfactory and nasal trigeminal nerves.

The olfactory area surface is - in the dog, at least - larger than the combined surface areas involved in seeing and hearing. The size of the area varies with the species of animal, ranging from 10 cm² for man to 180 cm² for the sheep dog. The olfactory area has a yellow color in many species, and it has been suggested that Vitamin A or carotenoids are essential components in olfaction, but a number of animals are not carotenoid storers and do not have significant concentrations of carotenoids in the olfactory area. Histologically, the olfactory area consists of receptor cells and supporting cells. There are vast numbers of receptor cells, varying from 10 million in man to 200 million in the dog. The receptor cells are topped with cilia that float in the mucous layer of the nasal cavity. It was thought that the cilia were involved in olfaction, but it has been shown that responses to stimuli occur even when 90% of the cilia have been removed. A nerve fiber goes directly from each of the receptor cells to the olfactory bulb.

Odors are important - not only physiologically but psychologically as well. It is possible to change the emotions with aromas, as in a cat's reaction to catnip. Physiologically, pregnancy in mice has been blocked by the introduction of a strange-smelling male into the cage of a female.

Odor quality is dependent to some extent on the structure of the chemical molecule. Introducing -CH₂ groups into the chain of the higher aldehydes, for example, can change the aroma of the compounds: C₁₄ smells like peaches, C₁₆ like strawberries, and C₁₈ like coconuts. Amoore has proposed a theory of olfaction based on chemical structure. Parosmias, in which there is a lack of response to individual compounds, are relevant to this problem. These compounds include: farnesol, methyl ionone, thymol, benzyl salicylate, ambergris, eugenol, steroid musks, HCN, and butyl mercaptan. Parosmias to the last two compounds have been reported independently by different workers.

Nerve impulses from the various parts of the system can be measured with electrodes, and the general form of the pulses can be differentiated. Inhalation of vapors of stimulants leads to the formation of spatial patterns of outbursts of electrical activity as the receptors respond. In order to obtain information about the pattern of response to

various stimuli and to determine the degree of response, a number of electrodes were implanted surgically in various parts of the olfactory system of a rabbit. Initially connected electrically through preamplifiers to audio and oscilloscope systems, the responses could be monitored while the animal was restrained. Recently, using telemetry to transmit the signals, it has been possible to study the behavioral response of the animals to the stimulus and record the electrical impulses simultaneously. The advantages of this technique lie in that long term studies can be made on the same preparation and that simultaneous responses can be obtained from a number of sites.

The responses of sixteen electrodes to a variety of odorants presented to the rabbit were photographed as they appeared on the oscilloscope screen. The location and intensity of the dots indicate the position and degree of response of the group of receptors near the electrode. By plotting the responses as shadowgraphs, profiles of the receptor activities can be obtained.

It has been determined that the receptors may respond with varying degrees of intensity to many stimuli; thus the receptors are probably not specific. Each receptor peaks at a different concentration, thereby giving a series of curves which the brain averages to obtain the response. The position of the receptor also seems to play a role in the response to the stimulus. Thus the pattern of responses of the large number of receptors in the olfactory epithelium may be necessary to differentiate the odors and the nuances in aromas of the various compounds smelled.

A CRITIQUE OF ODOR THEORIES

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Odor theories are supposed to describe how odors influence the activity of the olfactory area (how they are sensed) and how odors are differentiated. Unfortunately only tenuous hypotheses exist to explain the primary receptor process. Consequently, two trends are observed in the development of olfaction theories. Some researchers postulate a mechanism and search for correlations between the odor parameters and odorant properties that would be consistent with their hypothesis. Other researchers concentrate on the correlations in search of rules to predict odor parameters from objectively measurable properties of odorants. None of the present theories has even attempted to deal with all the odor parameters, quality, threshold, or intensity, and only a few have dealt with two at a time. Other odor parameters such as adaptation, cross-adaptation, and recovery rate have received little attention.

Most theories can be criticized for insufficient definition of quality parameters. The trend of olfaction theories is away from the concept of primary odors, as evidenced by the impossibility thus far to simulate any chosen odor by a mixture of odorant vapors that are not components of the chosen odor.

Most often odor quality is expressed as a point with characteristic coordinates in odor classification space. This approach with results leading in a similar direction is taken by many psychophysicists. The factor analysis method is used in addition to the older method of verbalization and odor attributes are obtained by: (1) semantic ratings, (2) ratings by comparison with a set, (3) direct similarity/dissimilarity, and (4) sensory titration. An example of the odor classification space by direct rating between various odorants (number 3, above) has been used by Woskow. We have prepared a model of the first three coordinates of his odor space, the configuration of which shows the distances between points for different odorants as approximately proportional to the judged difference in odor.

The number of coordinates or dimensions in the odor space that is required for proper classification of odor varies according to the level of sophistication. When rating is done by inexperienced observers or with a simple semantic reference scale, three dimensions are sufficient. With expert observers and with a more complex rating system, more dimensions are needed. Jones, working with expert perfumers, calculated that nine coordinates can probably match the information capacity of the perfumers' odor sense.

Olfaction theories should not overlook odor intensity. The most suitable reference for odor intensity is Stevens' equation:

$$\text{Intensity} = K [(\text{concentration}) - (\text{threshold})]^n$$

where $n = 0.2 \text{ to } 0.7$

In a consideration of various olfaction theories, the properties of odorants that have been pointed out relating to thresholds or type-directed qualities are molecular characteristics, combined molecular characteristics, bulk properties, and interaction with analogs. The molecular characteristics include size, shape, and spectra. The combined

molecular characteristics are shape and functionality. The bulk properties, such as molecular volume and vapor pressure, are in essence a physico-chemical shorthand for various combinations of molecular properties. Interactions with analogs, although derivable from molecular properties of the odor and the analog, describe the behavior of odorants with respect to other physico-chemical systems. The merit of the latter system is that, by bringing the odorant in contact with a variety of systems, some of them can be found to act as analogs for the odor receptors in the nose, and something will be learned of the properties of receptors.

Theories which advance correlations between objectively measurable properties and odors and, at the same time, attempt to hypothesize the mechanisms of the primary receptors in the nose are those of Davies (Mullins), Wright, and Dravnieks. Davies proposes that the adsorption-desorption process in the receptor membrane results in odor sensing and that adsorptivity at the water-lipid interface is one of the important factors. Mullins' theory which proposed that the odorant disorganizes the receptor membrane is a less elaborate precursor of the Davies theory. Wright proposes that a chemoreceptor substance or pigment is manipulated by the presence of odorant molecules between excited and de-excited states. Dravnieks has recently proposed an electrical effect theory which might be considered a derivative of the Davies theory. The criticism of these theories can be directed toward a lack of direct evidences since none of the receptor theories has been proven at the actual receptor level.

Davies' theory can be criticized in one instance for lacking sufficient number of dimensions. Wright's work dealt primarily with demonstrating that for some odorants within an odor class, certain far-infrared frequencies occur. The arguments which can be cited against this theory are: (1) Dermerdache has shown that frequencies considered characteristic are due to certain common molecular segments and similar frequencies can occur in molecules with different odors. (2) No effort has been made to find how many substances exist with similar characteristic frequencies but different odors. (3) For some substances with similar odors no characteristic frequencies have been found. (4) The intermolecular interaction increments that can be derived from the characteristic frequency with these low wave numbers are unlikely to be seen by any mechanism in the presence of much larger energy differences caused by other molecular features.

Nevertheless, in principle there should be some correlation between certain spectral features and odors, since presumably correlations exist between odors and structures, and the various spectra describe structural features. Such indications have been found by Michels and Wright, who factor-analyzed 50 odorants and showed some correlation between the psychophysical coordinates of odors in odor space and some features of spectra. Generally, however, there seemed to be no indication that molecular or intramolecular vibrations as such are causes of odor quality.

Dravnieks studied adsorption of odorants on various biochemical substances and the changes in the electrical potential of the surface of such substances when odorants were adsorbed. The substance that behaved most effectively was flavin mononucleotide. Two correlations were made. First, the odor intensity of undiluted odorants correlated with the electrical effect on adsorption, and secondly, the higher the adsorptivity, the lower was the threshold. Some objections have been raised to this theory on a biochemical basis. Of the theories based on mechanisms and physico-chemical correlations, Wright's and Dravnieks' propose a receptor substance, while Davies' does not. Davies, however, assumes that viscosities and surface tensions of receptor membranes can differ and provide specificity.

Theories which do not propose a mechanism but are based only on physico-chemical considerations can be divided into two groups. Wright, Amoore, and Beets' theories are based on inspection of molecules, whereas Laffort, Davies, Rosano and Friedman, Tanyolac, and Dravnieks propose comparisons of interactions with real systems. Davies' theory proposes interaction with an interface, Dravnieks, interaction with flavin mononucleotide, Laffort, solubility in water, Rosano and Friedman, interaction with cephalin monolayers and Tanyolac, interaction with organic or inorganic film systems.

The size and shape theories of Amoore and Beets are not pure steric theories. Both consider the influence of functional groups as well. Amoore's theory has a built-in provision for nucleophilic and electrophilic functionality, while in Beets' profile functional group theory one of the functional groups dominates and determines the position of the molecule during inspection.

Several difficulties occur with explanations of odor qualities by size, shape, and functionality alone. One is that size and shape can change continuously for a series of odorants, while the odors show discontinuities. This is not a criticism of Beets' theory, since it is easy to see how minor changes in shape or some functional group can change the oriented shape of the molecule. Size and shape are related to adsorption and are important in formation of donor-acceptor interactions but account only for some physico-chemical concepts. Consequently, theories based primarily on size and shape are limited. The common criticism to the shape, size, and functional group theories is that they pay too much attention to some causes of intermolecular interactions that are important at the receptor level but neglect others just as important, and sometimes critical. Of the various types of intermolecular interactions (dispersion forces, dipoles, hydrogen bonding, ionic and charge transfer) only dispersion forces are covered by shape and size factors.

In future considerations the physico-chemical properties must be considered in terms of a breakdown into force types or in terms of interactions with analogs on both. Odor parameters should be soundly based on modern psychophysical concepts such as odor space and directional concepts of classification. In comprehensive odor theories, odor quality, threshold, and intensity should be considered together.

THE FLAVOR PROFILE

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The flavor profile is a taste-panel method, worked out at ADL, for specifying flavor factors (taste, aroma, and feeling) of a material. The complete profile gives (1) the whole impression, (2) the detectable factors, (3) their intensities, and (4) their order of detection.

Introduction

Flavor is all sensations of taste, smell, and feeling when food is being eaten. Flavor results from the chemical stimulation of the taste buds in the mouth, the olfactory end organs in the nose, and the end organs of feeling in the mouth, throat, and nose.

There are only four tastes: sweet, sour, salty, and bitter. These are often termed basic or primary taste factors, since physiologists have shown that only these will stimulate the taste buds. No such clear-cut limitations apply to the chemical stimuli of the olfactory organs. People have remarkable memories for many, many odors. This implies that there may be several hundred thousand odor stimuli, but how many of these are released by foods is not known. Then there are the chemical feeling factors, less well-defined physiologically than odor factors. Examples are astringency (alum), cooling (menthol), bite (pepper), and burn (horseradish).

Flavor identity of a food depends upon the concentrations of the specific chemicals released during eating and the specific sensitivity of the human organism. Foods may have many chemical components, but not all will be individually sensed. For example, brewed coffee flavor is readily recognized, but one can only taste bitter and sour, feel astringency, and detect "bouquet." In the coffee flavor profile, the palm of the hand represents the unidentifiable chemicals (which do create a flavor impression, for without them coffee is not coffee); and the fingers represent the bitter, sour, astringency, and bouquet factors. The whole hand represents the whole flavor.

The concept that food flavor is a complex of both unidentifiable and identifiable chemicals applies to food aromas and to perfumes as well. For example, a fairly simple perfume can be made from a mixture of a few essential oils, resins, extracts, and aromatic chemicals. The perfume's aroma is unquestionably fragrant, but even those who know the kinds of chemicals likely to occur in the ingredients cannot say, "I smell such and such terpenes, polymers, aldehydes, and esters." About the most they can say is, "This is a heavy aroma, floral in nature, with a slightly pungent top note redolent of a higher aldehyde."

Specifying Flavor

A flavor profile, whether written out or presented visually, should give quantitative, as well as qualitative, information. Much has been published on the sensitivity of the human senses of smell and taste for specific chemicals, and some of the values are astounding. The odor threshold in parts per million of air for propyl mercaptan is 0.000075; for coumarin, 0.00034; for hydrogen sulfide, 0.0005; for ammonia, 50. Taste thresholds for common chemicals in water, given in percent by weight are: sucrose, 0.34; salt, 0.04; citric acid, 0.004; and quinine sulfate, 0.0002.

Threshold measurements are the surest ones available, because they refer to the lowest concentration at which a chemical is recognized. The threshold levels for different individuals differ, but their impressions of increasing concentration may be reported as identical because individuals cannot, with precision, "measure" taste intensities beyond threshold, unless they can compare test materials against standards. Thus, two individuals whose thresholds for sucrose may be 0.34% and 0.40% may both report a 10% sucrose solution as "moderately sweet." Taste experiments conducted in Vienna in 1908 showed that people can detect 25 "just noticeable differences" (JND's) in sucrose solutions ranging from threshold to strong in intensity. Odor experiments conceived by R. M. Hainer and conducted at ADL show about the same number of JND's for anethole in mineral oil.

One more aspect of flavor which has to be considered is its duration. When a bite of food is eaten or a sip of beverage drunk, the entire sensation of flavor usually lasts only a few seconds. However, some foods and beverages, even after being completely swallowed, leave a flavor residue termed aftertaste. The aftertaste, incidentally, is not a miniature of the whole flavor but rather just the delayed effects (such as astringency and dryness) or long-lasting factors (such as bitterness).

Each detectable flavor factor of a food has its own time-intensity curve. Most flavor sensations build up rapidly to a peak and then fall off rapidly. Thus if intensity is plotted along the y-axis against time along the x-axis, the flavor sensation curve looks like an arch, and a plot of all detectable flavor factors will look like an uneven fence. Such a plot suggests that different flavor factors may be sensed separately and at different times, and they are, although the time span may be only microseconds. Figure 1 shows an example of the time-intensity relationship of flavor perception as worked out for beer.

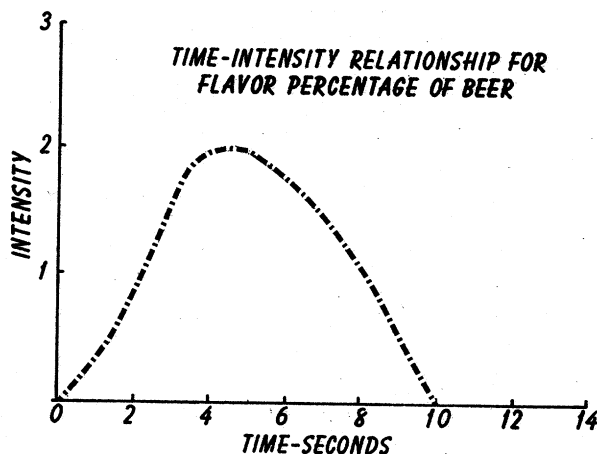


Figure 1

Flavor Profile Panels

A flavor profile is always worked out first in words by a trained flavor panel. The flavor analyst should be allowed to concentrate wholly on his unknown. So he is provided with a place to sit and a table at which to work in a quiet, well-lighted, odor-free area, and his work is scheduled. The plates and utensils he uses are not only clean but odor free. The temperature of his sample is the same as that of other panel members' samples, and it is the same from day to day. Furthermore, he and his analytical conferees all examine their samples in the same way - the same number of sniffs for aroma, the same number and size of bites or sips for flavor - at the same time and at the

precise temperature selected for the product being tested, since flavor properties will be different at different temperatures. All the panel members communicate their flavor sensations verbally, and they all use the same descriptive terms.

That all panel members use the same terms presupposes a common experience of expressing their sensations. This is attained first through general training in odor and flavor, but then through specific practice with the product at hand. Thus, each term listed in a profile tabulation is understood by all the panel members. Qualitative factors are

defined in terms of reference materials. Obviously, "sweet" taste is so common that it need not be referred to sugar. But "red candy flavor" can be referred to cassia, strawberry, raspberry or anise; rancid fat can be defined as three, six, or nine months old by using reference fats of known ages.

When the qualitative terms are defined, assignment of numerical intensity values becomes easier. The intensity scale is threshold, slight, moderate, and strong, and for convenience, these levels are given as: 1, 2, and 3. All but threshold are ranges in themselves, and experienced panels can specify more precise values by using reference intensities. For example, a series of known concentrations of sugar solutions will provide one solution that matches the sweetness of a test fruit juice. Thus, the panel may agree that although the sweetness of both juices A and B could be rated at the intensity of 2, juice A was sweeter because it matched a 14% sugar standard while juice B matched a 12% standard. Or the two juices could have been tasted side by side, and the higher sweetness of A would have become evident.

Ordinarily profile analyses are not conducted side by side. Temperature control is difficult, physiological fatigue of the taster occurs, and lasting after-effects interfere. Some single flavor components such as sweetness can be studied side by side. But because of lasting effects, two bitter samples cannot be intercompared, nor can a glutamated sample be compared with its control.

In panel work, aroma of the sample is examined before flavor, because its profile will include odor notes which may be overpowered when the food is eaten. For example, a cold cereal may have a waxy odor in its aroma, but it may not be perceived when the cereal is eaten because other flavor notes are apt to drown it out.

Following aroma examination is flavor analysis. Flavor-by-mouth, as it is called, is the specialists' description of sensations that can be perceived by consumers - the taste factors, the aromatic factors, the feeling factors, and the aftertaste - the sensations through which the consumers decide whether they like a product, and if they like it well enough to buy it again. Table I shows the flavor profile as worked out by our flavor panel for Chip-O's.

Table I

Flavor Profile for Chip-O's

<u>Aroma</u> <u>Characteristic</u>	<u>Intensity</u>	<u>Flavor-by-Mouth</u> <u>Characteristic</u>	<u>Intensity</u>
Sweet	1-1/2	Sweet	1
Toasted corn	2	Toasted and slightly burnt corn	2
Oily	1	Limed corn	1
Briny	1/2	Oily	1
		Salty	1-1/2

An effective profile panelist should be so familiar with terminology that in, say, three sniffs and three bites, he can write down accurate word descriptions of aroma and flavor;

what was the overall impression or amplitude? Was it low, medium, or high? What were the discernible factors? How strong was each? Which came first, second . . . last? What was the aftertaste?

The final product of panel work is a complete flavor profile, with both the aroma and flavor-by-mouth described and specified as accurately as possible. In summary, it consists of (1) the whole impression, (2) the detectable factors, (3) their intensities, and (4) their order of detection.

The Method at Work

Psychologists classify the flavor profile method as a phenomenological one, as opposed to psychometric and psychophysical methods. Psychometric taste tests are exemplified by difference tests which determine the number of persons finding a difference between samples. Psychophysical tests measure the reactions (like and dislike) of tasters to one or more samples. We found that neither psychometrical nor psychophysical tests provided the kind of information needed to define the role of monosodium glutamate in food flavors. Nor would they have been adequate for our succeeding problems. But we have found that, through flavor profile analysis, it is possible to define food problems, measure the success or failure of small and large technological changes in formulation and production, interpret consumer reactions to foods, and design new products.

TASTE PANEL METHODS OF FLAVOR ANALYSIS

Amihud Kramer
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Definition

Any discussion on flavor first requires a definition of just what is to be included under this general term. You will note that Dr. Moulton and Dr. Dravnieks limited their discussion to odor, while Mr. Kendall not only included taste together with odor but also gave consideration to what we might call "mouth-feel." There is general agreement that both taste, the responses of the taste receptors of the tongue and odor responses of the olfactory receptors of the nose are both legitimately and completely included under the general category of flavor. But there is considerable disagreement as to whether "mouth-feel" responses should be included in the flavor category, or, since they are primarily muscle-sense responses, if they should not be more logically included with texture, under another general category of "kinesthetics" or the muscle sense. Similarly, there may be some question as to whether "off-flavor" should be considered under the flavor category or under another category which may be called "defects," particularly if the defects are also visible and could, therefore, be classified under the category of "appearance." I would like to propose an open-end definition, as illustrated in the following diagram, where the flavor category blends on one side into the kinesthetic category, and on the other side into the appearance category. Directly and entirely under flavor I would place taste and odor. Intermediate between flavor and kinesthetics, I would place mouth-feel, and similarly in an intermediate position between flavor and appearance I would place off-flavor.

APPEARANCE		FLAVOR		KINESTHETICS
	Off-flavor	Taste	Odor	Mouth-feel

In this way, the definition of flavor could remain flexible, including, or omitting as may be appropriate in each particular case, certain attributes of mouth-feel and off-flavor. For the specific purpose of this discussion, I intend to consider flavor in the broader sense as did Mr. Kendall, including these peripheral areas, although the title of my presentation includes the term "taste."

There is no one taste panel procedure that is a best method for all purposes. Kramer and Twigg (1) classified taste panel methods under five purpose classifications. A committee on sensory evaluation of the Institute of Food Technologists listed ten types of sensory evaluation tests that would be used for different purposes (2).

Identification

A taste panel may be needed for identifying a specific attribute of flavor, or perhaps even a specific chemical component. For such a very limited objective, one very highly trained and skilled expert may be superior to a group, particularly if a known component is being sought. The only advantage of using more than one panelist for such a purpose is that a particular unexpected attribute may escape observation by a single expert, but such an error is not as likely to occur if several panelists are used.

If we should add an indication of intensity of one or more attributes or components to mere identification of their presence, we have the highly trained, reproducible flavor profile method as described by Mr. Kendall.

Differentiation

In many instances the purpose of the panel may be not so much to identify certain attributes of flavor, but to detect or perhaps measure the difference among two or more samples. Such a procedure carries with it a tremendous advantage, since psychologically the human taster can always judge much more precisely by comparison than by absolute judgment. For purposes of determining differences, training is useful but not as important as in identification; nevertheless, a small panel is usually adequate. A number of taste testing procedures such as the paired comparison, duo-trio, and triangular have come into general use. Unfortunately, these methods are "attributes" procedures which have low statistical power. Therefore they require considerably more work, that is, replication, to achieve the same accuracy as "variables" methods. This is due to the fact that these attributes procedures merely indicate whether there is or is not a difference, but do not indicate the intensity of the difference or the direction of the difference.

Difference-Preference

A difference-preference panel can be used in most instances to obtain answers as to whether two or more samples are different, and at the same time provide some indication of preference with much less effort in terms of number of tastings and/or panelists if a multiple comparison method is used. By this method the samples that are compared are not limited to two. The number of panelists can be as small or smaller, and the number of replications can be reduced very substantially and still achieve equal accuracy. The major difference between this "variables" type of test and the "attributes" type of test is that the multiple comparison method utilizes quantitative scalar data and can at the same time indicate a degree of preference as well as difference. For such purposes a balanced scale is particularly useful. Thus, for example, for the purpose of determining an optimum sweet-sour (sugar/acid) ratio, a sample which is neither too sweet nor too sour may be scored as zero. Increasing positive values may be used to indicate increasing levels of sweetness, while increasing negative values may be used to indicate increasing levels of sourness.

Preference

Finally, we come to a strictly preference, that is, consumer-type panel. Where the purpose is to estimate consumer preference, none of the above procedures are adequate, except perhaps the difference-preference multiple comparison procedure, and that only as a crude indication. Panelists not only need not be trained but should not be trained. To achieve more than crude results at least fifty panelists should be used, but there is generally no real advantage in using more than about two hundred panelists. Of course, the panelists should be selected to represent the target population. Obviously, it would be a folly to select school children as panelists if different kinds of beer are evaluated, or to select a panel of American housewives if flavor differences among different kinds of soy milk intended for use in Southeast Asia are to be evaluated for preference.

If different formulations of a new or conventional product are to be evaluated for preference by the general population, the panelists should be selected and identified as representing different population groups by age, sex, ethnic or geographic origin, etc. If groups of

panelists are so identified in advance, then not only general preference for a particular flavor can be established, but a statistical analysis of the results may determine if there is a significant interaction between panel groups and flavors. This could lead to most important conclusions, such as one flavor being preferred by one group of consumers, and another flavor being preferred by another group of consumers.

Relation of Specific to General Attributes of Flavor

It is frequently necessary to use several types of panels at different stages in the progress of the development or improvement of a product. Thus, for example, in the earlier stages of product development, one or more formulations or processes can be evaluated by a small difference-preference panel for specific attributes or components, while at a later stage of development it may be found necessary to do consumer acceptability studies per se as well as to relate the specific attributes of quality (as determined by objective chemical or instrumental methods, or by taste panels) to the overall quality as determined by a consumer preference panel. If such data are numerical and quantitative, then a multiple regression analysis can be performed to determine the relative importance of each specific attribute in the overall evaluation, with the multiple regression equation indicating an optimum level for each of the individual components for maximum consumer acceptance. In such regression analyses, it is important to recognize the probability of certain non-linear relationships. If the samples examined are all well within acceptable commercial range, deviations from linearity may not be important. If, however, some of the formulations include extreme values, quadratic or cubic transformations should be made before routine multiple regression analysis is performed.

Conclusion

In conclusion, I would like to re-emphasize the importance of knowing exactly the purpose of the taste panel before selecting the type of panel to be used. If the purpose is to identify, then the emphasis should be on the use of a few highly trained panelists. If to differentiate, consider the advisability of using a difference-preference multiple comparison panel that will provide more accurate difference data as well as some indication of preference. If to determine consumer preference, only a large unskilled panel of consumers can provide the answer.

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CHEMICAL METHODS OF FLAVOR ANALYSIS

Irwin Hornstein
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Thousands of kilograms of fruits or vegetables are sometimes processed to obtain milligram amounts of oils, containing perhaps microgram amounts of organoleptically important materials.

Concentration techniques are obviously important. Distillation, extraction, crystallization, and lyophilization may be used. This concentration step to be meaningful must be accomplished without producing artifacts. (In distillation the greatest problem may be heat damage to volatiles and in extraction the introduction of contaminants from solvents.) Care must be exercised to avoid introducing volatile chemicals from containers, tubing, lubricants, plasticizers, etc.

Once the concentration step has been accomplished, gas-liquid chromatography (GLC) is the separation technique of choice. Separations on at least one non-polar and one polar liquid phase should be attempted. In addition, because of the wide range of boiling points, the separation of the compounds in these mixtures is made using temperature programming. The choice between capillary and packed columns will depend upon the complexity of the mixture. The large number of theoretical plates associated with capillary columns is somewhat misleading. To provide the same resolution, capillary columns, because of their much higher ratio of gas volume to stationary phase volume, require a larger number of theoretical plates than a good analytical packed column with a lesser number of plates. If a packed column with 5,000 to 10,000 plates provides satisfactory separation, there is no reason to use capillaries. The 0.01" capillaries, in particular, are difficult to make, require careful handling, and can be easily overloaded and rendered useless. If capillaries are used, a choice can be made among 0.01", 0.02", and 0.03" I.D. columns. A 200-foot 0.01" column can handle small amounts, about 1 μ g, of material per peak. A 500-foot 0.02" capillary can handle between 10 and 20 μ g per peak and is a good compromise between quantity and efficiency. A 1000-foot 0.03" column can handle between 100 and 200 μ g per peak and compares in capacities with 1/8" packed columns.

Cryogenic temperature programming has received considerable attention in flavor research: programming can start at -100°C and can continue up to 100°C or more. The initial temperature in a programmed run will not improve the separation of higher boiling compounds, but the separation of low-boiling compounds is improved immensely. Just how important these low boilers may be is probably a function of the food or problem being studied. A preliminary separation evaluating the organoleptic importance of the low-boiling fraction should be made before deciding upon cryogenic programming.

A flame ionization detector is usually the detector of choice. On the plus side, the detector is simple in construction, extremely sensitive to organic compounds and insensitive to water; on the minus side, the compounds are destroyed on passing through the detector. If the separated compounds are to be further studied the effluent from the GLC column must be split, part going to the detector, part to the observer, instrument or trap.

Once the components in a mixture have been separated their identity must be determined. Compounds coming off a column can be trapped and examined at leisure or

scanned on the fly. Information concerning structure may be obtained from retention data, usually insufficient to provide unequivocal identification; instrumental methods of identification are most important. The combination of GLC and mass spectrometry yields the greatest structural information, since (a) the amount of material required for a mass spectrum compares favorably with the amount detected by GLC, and (b) scanning the effluent from a GLC column without trapping is a practical reality. Unequivocal identification of all compounds by mass spectrometry is not a reality. In many instances additional information is required. Infrared, Raman, and nuclear magnetic resonance spectrometry are instrumental methods that can supply additional structural information. However, in order to use these techniques samples must be trapped since large amounts of a compound are required. IR can be pushed down to the microgram level if microcells, KBr micropellets, beam condensers, and scale expansion are used. Raman spectrometers, using a laser source, can be used if approximately 100 micrograms of material are on hand. NMR is undoubtedly the least sensitive of the spectrometric methods. However, practical microcells and electronic devices which improve signal-to-noise ratio have become available. At present, with time averaging and a 50-microliter cell the minimum sample size may be 10 to 25 micrograms.

Chemical methods such as functional group analysis can also supply useful structural information. Hydrogenation, hydrogenolysis and pyrolysis of isolated compounds, and examination by GLC of the products produced by these treatments also supply useful structural information.

The flavor chemist, even after identifying the compounds he has isolated, has however not solved his problem. He must further evaluate the organoleptic characteristics of his isolates. To a first approximation it is reasonable to assume that if a compound is present in the volatiles at a concentration greater than that required for detection, it must exert some influence on the flavor. Greater emphasis is now being placed on determining olfactory thresholds. Attempts are also being made to evaluate the organoleptic significance of compounds by computer techniques. These approaches are in their infancy but promise to be the exciting areas of future flavor research.

there is no commercial production in this country. These compounds are usually prepared in the form of a 50:50 mixture of the two, but are also available individually, and in combination with MSG. They are relatively expensive, but the price situation is steadily improving and has changed from about \$40/lb. a short while ago to about \$20/lb. or lower today. Fortunately, the quantity of nucleotides required for desired flavor effects is quite low, and usually ranges from 0.001% to 0.1%.

In general, the 5'-nucleotides have produced beneficial flavor effects in soups, gravies, bouillons, certain canned vegetables, and certain canned meat and fish products. They may be used in partial replacement of beef extract and to suppress undesirable heat-induced flavor notes in hydrolyzed vegetable protein. Because of their synergistic reaction with MSG, they are especially useful in many food systems containing added glutamate. This MSG-sparing effect can be very important to food manufacturers as a means of reducing production costs.

In this regard, we studied the MSG-sparing effect recently in six different commercial canned and dry soups. In each instance we found that a significant sparing of MSG could be achieved by use of appropriate levels of IMP:GMP in conjunction with lower levels of MSG (as compared with normal use levels). The MSG-sparing effectiveness of the mixture of 5'-nucleotides varied with the food product and according to the concentration ratios with MSG employed (Figure 3).

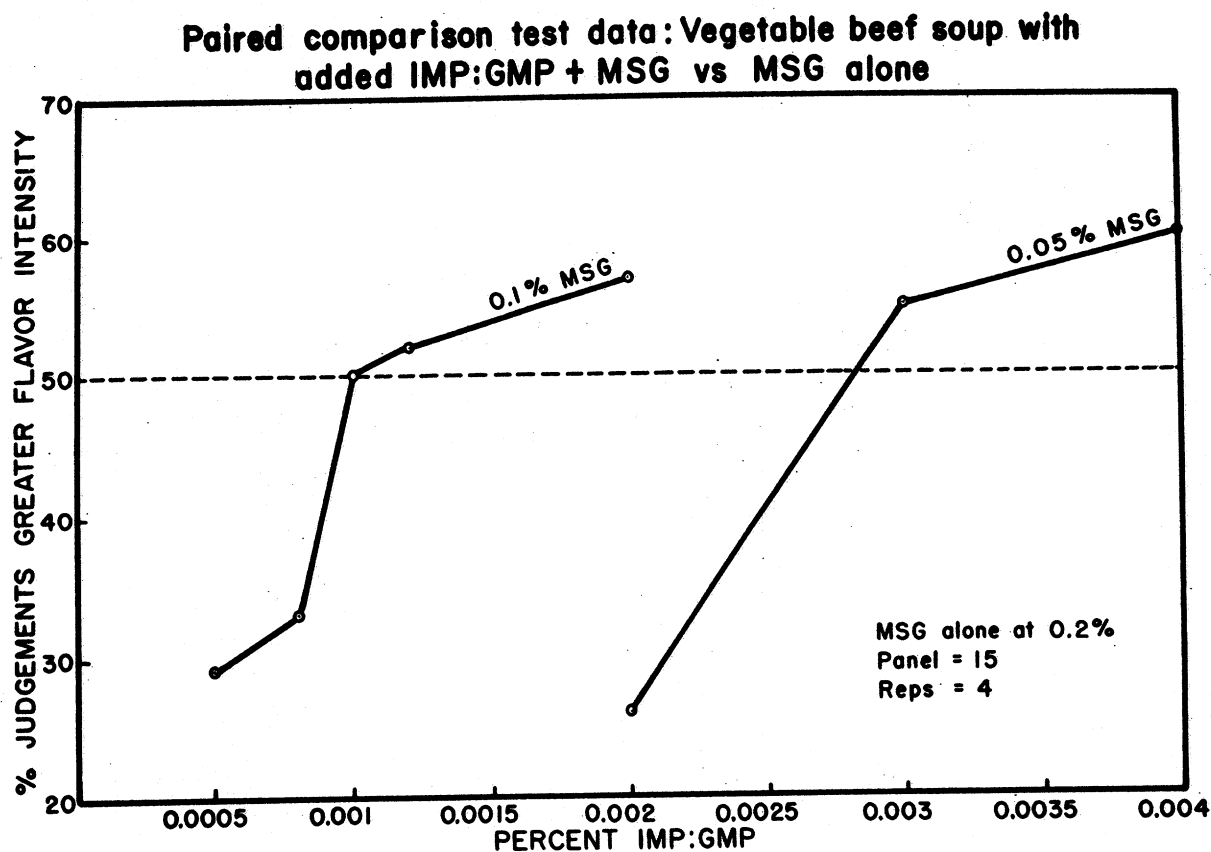


Figure 3

Other applications of nucleotides have been mentioned in the literature, but I think we have considered enough for the present. I believe a word of caution is needed at this point with regard to the use in food systems of nucleotides or any other kind of flavor potentiators. We know that in some instances they may suppress certain flavor notes (e.g., sulfur notes, metallic taste, bitterness, etc.) and that others may be enhanced. Some preliminary work we have done indicates that the sweetness or bitterness of saccharin may be enhanced depending on the concentration of IMP:GMP used. Since one fraction of the flavor complex may be depressed while another may be augmented, the result could be a serious imbalance in the total flavor impression of the product. We need much more information on the nature of the interactions of these materials with food flavor constituents.

FLAVOR DEVELOPMENT BY BIOLOGICAL MEANS

Phillip Issenberg
Massachusetts Institute of Technology

Most natural food flavors are products of plant, animal, or microbial metabolism. Current knowledge concerns three important methods for developing volatile flavors: culture of microorganisms (beverages, cheese); extracellular enzymatic reactions that occur after the structure of the intact fruit or vegetable is disrupted (onions, cabbage, certain other fruits and vegetables); and flavor production during ripening processes in the intact fruit after harvest at maturity (bananas).

A large body of biochemical knowledge has been developed concerning pathways for formation of volatile flavor compounds by microorganisms. This is due largely to the ease with which pure cultures may be grown on synthetic substrates under controlled conditions. In addition, auxotrophic mutants of the microorganisms having specific pathways blocked are often available. These factors greatly simplify the study of metabolic pathways. Analogous pathways probably exist for the formation of these compounds in higher plants.

Most studies of production of volatile flavor compounds by microorganisms have been concerned with the flavors of fermented beverages. A large number of volatile components have been isolated from fermented beverage and food products. These include alcohols, acids, esters, aldehydes, ketones, lactones, and other classes of compounds. Some of these are produced during the fermentation and others are present in the starting material.

A summary of major pathways contributing to synthesis of higher alcohols by Saccharomyces cerevisiae shows that all of the alcohols can be produced from carbohydrates. Straight chain alcohols may be produced by acetyl-CoA coupling, with the aldehydes as penultimate intermediates. Transamination of amino acids to the corresponding alpha-keto acids is involved in formation of the branched chain alcohols (isobutyl, isoamyl, active amyl), and n-propanol. All of the corresponding aldehydes are found as intermediates and may also have flavor significance.

The acid analogs of the aldehydes and ketones may be formed directly from the aldehydes through action of aldehyde dehydrogenase in the presence of NAD, or from ketones by decarboxylation of the alpha-keto acid. The latter reaction involves alpha-keto acid oxidase, NAD, and CoA, and results in formation of the acyl CoA.

The major pathway for formation of esters involves reaction of acyl-CoA with alcohol rather than direct combination of free acid and alcohol. Additional acyl-CoA, representing longer chain acid residues, may be available from synthesis of higher fatty acids. Apparently rather non-specific enzyme systems affect reaction of acyl-CoA with a variety of alcohols. For production of acyl-CoA directly from free acids, ATP is required as an energy source.

Lipid metabolism by microorganisms also results in the production of volatile compounds. A cheese mold, Penicillium roqueforti converts the common even-carbon-number fatty acids present in milk lipid to methyl ketones containing one less carbon atom. P. roqueforti spores and mycelia convert the ketones to the corresponding

secondary alcohols. Similar reactions were found in a variety of microorganisms. Ketones and secondary alcohols appear among the volatiles isolated from higher plants, and may be formed by analogous pathways. However, caution must be exercised in extrapolation of these relatively well characterized pathways to the metabolism of higher plants.

There are two areas of special interest in production of flavors by extracellular reactions. One is the production of volatile flavor following slicing or crushing of materials which contain low levels of volatile flavor compounds prior to tissue damage. The other is the regeneration of flavor lost during dehydration or other processing by enzymatic conversion of stable flavor precursors to volatile compounds.

The members of the onion genus (*Allium*) have very weak aromas in the intact state, but develop strong pungent flavors when the tissue is damaged by slicing or crushing. This is due to formation of volatile sulfur compounds, thiosulfinates, from non-volatile precursors, S-substituted cystein sulfoxides. The thiosulfinates are unstable and in turn may decompose, producing disulfides as breakdown products. Both thiosulfinates and disulfides have strong odor properties. Differences in aromas of the common *Allium* species (onion, garlic, leek, and chive) may be due to quantitative differences in the concentrations of the various sulfur-containing volatile constituents, which may reflect quantitative variation in the non-volatile components.

Enzymatic regeneration of volatile flavor compounds lost during processing is exemplified in the formation of flavor in dehydrated cabbage by addition of enzyme preparations from cabbage and mustard. This results from the formation of isothiocyanates as a result of enzymatic action on thiohydroximyl glucosides (present in cabbage and other *Brassica* species). Distinct flavor changes induced by treatment with enzyme preparations have been observed in processed cabbage, horseradish, peas, beans, carrots, tomatoes, and onions. Raspberry aroma was produced from unripe raspberries following treatment with an enzyme preparation from ripe raspberries.

The flavor of many fruits depends upon the presence of a large number of volatile compounds of different types. These are produced over long periods of time and accumulate in the tissues during maturation and ripening.

Bananas are mature when picked, but possess very little aroma. Almost all of the characteristic flavor is developed as the fruit ripens during storage. Esters and alcohols seem to have major flavor significance. Many of these components could be produced by the same pathways which have been elucidated in yeast, although an even greater complexity of metabolic relationships can be expected in ripening fruit.

Isoamyl alcohol and isoamyl acetate are important components of banana aroma and were selected for study. Possible precursors of these compounds, labeled with radioactive carbon, are being administered to fruit slices and the components of the volatile emanation examined for the presence of the radioactive carbon. Preliminary results of these experiments and of experiments employing enzyme inhibitors were reviewed.

FLAVOR DEVELOPMENT BY PROCESSING

R. E. Moores
General Foods Technical Center

The development of flavor during food processing involves many reactions and mechanisms. Among the most significant flavor-producing reactions are: sugar caramelization, Maillard-type reactions of sugars and sugar decomposition products with amino acids and protein products, decomposition of lipids, phenols, nitrogen ring compounds and interactions of these substances. The number of compounds contributing to flavor generally increases with higher temperature and longer processing time.

The chemistry of flavor development during cooking, baking, and roasting appears in the flavor of cooked potatoes, baked bread, toasted cereals, and roasted cocoa and coffee.

Methods and instruments for isolating and identifying flavor components in processed foods have advanced rapidly in recent years. Good qualitative data, especially on volatile flavor compounds, are available. Reliable quantitative data are scarce and difficult to obtain. Advancing knowledge about the chemistry of process-derived flavors, better objective and subjective methods, and skilled artistry of flavor chemists are contributing to better quality and uniformity of foods and to greater production efficiency.

SYNTHETIC FLAVORS FOR NEW FOODS

Harry Fields
International Flavors and Fragrances, Inc.

In order to determine current food trends we must first understand some of the forces leading to food innovation. One of these is the rapidly increasing world population, requiring increased efficiency in food production. The earth's population will double in 30 years. What will they eat?

Another factor is the increasing affluence in many countries of the world, giving rise to a desire for convenience foods - a sort of built-in maid service. This, in turn, creates a demand for food ingredients with special functional properties. Also, increased labor costs have forced further mechanization of food manufacture, which has accelerated mass distribution. The changing modes of living exert their influence as well. There are greater non-domestic demands on the homemaker and, consequently, more away-from-home eating. Greater informality in family relationships, irregular hours, and more leisure time lead to fewer regular meals and more snacking. A reduction of physical labor reducing caloric requirements suggests a direction for new foods; so does more emphasis on better diet. There is an insatiable desire for variety on the part of consumers and a continuing drive by food manufacturers for new products yielding higher operating margins. This process is accelerated by ever-increasing knowledge of nutritional requirements and food technology.

Man's food habits change slowly; yet there are many indications of things to come in today's market place. Tomorrow's foods will include modifications of today's products, as well as truly new products. Practically all of these will depend in some important measure for their success on proper palatability and unique and pleasant flavor. Let me mention some examples of what I believe are things to come.

We visualize pre-packaged unitized entrees, side dishes and complete meals prepared in special heating units, utilizing radiant heat, high frequency energy and steam. These units will be a standard home appliance and will make it common practice for a typical household to offer a varied menu at any meal. It will also minimize the labor requirements of restaurants.

We foresee increasing varieties of established products, initially merely more esoteric variations of known products, but ultimately yielding completely novel formulations.

Also, technically sophisticated products with synthetic components, yielding end products similar to existing foods. Animal products will gradually be replaced by high protein foods from non-animal sources. The world cannot long afford to continue meeting its protein needs from inefficient and expensive animals. In the long run microbiologically and synthetically produced proteins and amino acids will probably be utilized, but in the next several decades these foods will be based on protein from fish, soy bean, and many other vegetable sources. In the near term we are likely to see products comprising both meat and non-meat protein-rich materials. In some cases these protein foods will be simple blends of vegetable protein-rich materials, in some cases quite sophisticated products, such as those made from spun protein. There will be a proliferation of products in which butter fat is supplanted, for example, by non-dairy creamers, whipped toppings, sour cream, mellorine, and non-milk protein beverages.

The decline of formal eating situations will give rise to a need for nutritionally balanced foods which are either ready-to-eat or very easily prepared. The jam filled toaster products and pronto pops of today are likely to be predecessors of a new genus of nutritionally self-sufficient foods. These products will provide the manufacturer with wide latitude in product design. We believe in a further evolution of today's snack products, resulting from decline of formal eating.

There will be an abundance of new type low-calorie foods incorporating synthetic, low-calorie sweeteners in addition to flavor and texture contributing components. The products will continue the hedonistic path charted by low-calorie beverages.

I would also like to mention proprietary entrees and side dishes, analogous to today's ready-to-eat breakfast cereals, as well as foods permitting a faster mode of ingestion, for example, instant breakfasts. In short, we will deal more and more with foods that have been subjected to processing of some sort, be it canning, freezing, irradiating, compacting, freeze drying, vacuum drying, puff drying, osmotic dehydration, freeze concentration, etc., etc. Help is obviously needed to make these foods taste the way Mother Nature intended. We will be increasingly concerned with restoring the former flavor levels of natural foods. When yields and tonnages per acre are dramatically increased by the farmer's use of ever more efficient chemical fertilizers and pesticides, and when growth is accelerated in cattle and poultry by "hurry up" feeding methods and diets, the natural flavor of the plant or animal does not have time to fully develop. Some completely synthetic foods will be entirely dependent upon flavor addition for their identity.

Now, what is being done to meet these flavor challenges? To begin with, we are seeing solid emphasis on synthetic flavors. This makes sense to the scientist to whom "synthetic" means more reliable, less expensive, more abundant, and usually better adapted to a special purpose. To the less well informed, however, it may suggest unnatural, less good, and even possibly dangerous. While the stigma once connected with synthetic flavor additives is disappearing fast in the United States, the same cannot yet be said in the case of certain foreign countries. But the use of synthetic flavors is not only inevitable, it is in most cases actually preferable.

The synthetic ingredients manufactured in our chemical plants are literally purer than natural flavor ingredients, the latter possibly containing objectionable trace impurities or highly volatile compounds which may evaporate, resinify, or oxidize. As a rule, synthetics are of greater intensity and therefore more compatible in the final flavor formulation. The flavorist need not contend with large extraneous quantities of liquid or other inert materials. They are generally less subject to bacterial contamination, less expensive, in virtually unlimited supply, and well adapted for use in modern foods.

Much has been said about developing synthetic flavors with greater fidelity to their natural counterparts. I believe it is fair to say that no natural flavor has as yet been perfectly reproduced. In some cases we are very close but in others perfection or even reasonable approximation still eludes us. Various techniques of instrumental analyses, of course, are very helpful in this direction and the gap is being steadily narrowed. But scientists also realize more and more that the proper use of identification data obtained by instrumentation generally falls outside their field of competence. The creative flavorist is best qualified to reassemble the constituents found by instrumentation. Optimum results are only attainable by very close cooperation between scientists and creative flavorists.

But it is not sufficient to create a synthetic flavor with great fidelity; the flavor must be delivered at point of use - that is, to the one who eats the product. Flavors that are delicious in a taste solution often come through badly after processing. Here a partnership of food technologist and creative flavorist is of the essence, calling for real craftsmanship in flavor formulation. How can we compensate for processing losses? Prevent the flavor from breaking an emulsion? From reacting with another food ingredient or a metal can? Making it water soluble or oil soluble; improving its performance under acid - or less acid - conditions; dealing with the bitter aftertaste of some synthetic sweeteners? Or transforming it into a powder or a paste; how to keep it from oxidizing, evaporating, diffusing through a polyethylene package, etc., etc. All these are complex problems deserving, but not always obtaining, maximum attention. My company has added many food technologists to work closely with basic scientists and creative flavorists, and I suspect this pattern will be further accentuated all through the industry.

Great strides are being made in particular in the area of powdered flavors. Spray drying - now almost universally used - has many well known drawbacks. The frontrunner among systems to supersede spray drying is the microencapsulation process by coacervation. After many years of often frustrating research, it is now a reality for at least some applications. We are confident that the remaining problems, for example, of how to make a cold water soluble capsule, will also yield to sustained efforts being made.

An interesting phenomenon in flavor development is the increasing preoccupation with what we call the "main part of the meal" flavors. The traditional research into fruit flavors - useful and needed as it is - is now overshadowed by inquiry into the flavor of meats, poultry, fish, dairy products, vegetables, and spices. It is spurred on by needs created by new processing techniques harmful to flavor, by the questionable economic outlook for some natural proteins and - last but not least - the advent of derived and synthetic foods.

Flavors are also needed increasingly in less developed areas of the world on a scale previously unknown. Since exposure to outside influence is minimal, the very poor are slow to change. Desperately hungry people in such areas will not eat nutritious food which is strange to them in flavor, form, or texture. The flavor preferences in Asia, Africa, and Latin America are unlike our own. Many favorite taste sensations in those parts of the world often are completely unacceptable to Western palates. Until recently this part of flavor research has been neglected and much still remains to be done. The dramatic effect of proper flavoring is illustrated by the sixteen different ghee flavors IFF has created to give Western butter the burnt and rancid taste demanded in India. Complicating matters further, flavors vary from region to region. A flavor we developed for UNICEF to be used in a liquid weaning formula was acceptable in Algeria but not in several other African countries. The durian fruit, a delicacy in Indonesia, is considered putrid in other parts of the world.

In the Western world, and particularly our own country, we see an increasing demand for more flavor variety, new modern taste sensations, and more innovation. It stimulates the creative flavorist to transform the highly personal concept of an artist into flavor reality and to do so with a high degree of practicality. But he can only succeed through partnership with the scientific community, profiting from the fine work being done in laboratories and seminars such as this one.

LIST OF ATTENDANCE

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ACETO, N.C.	Eastern Util. Res. & Dev. Div.	Wyndmoor, Pa.
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This conference on Food Flavors was one of a series of annual collaborators' conferences which are organized by the regional utilization divisions of the Agricultural Research Service, U. S. Department of Agriculture. The collaborators are staff representatives of the State Agricultural Experiment Stations in each of the four regions. To assure depth and breadth in subject matter, a single area of important research is selected for each conference.